

Decoration 'Decoration #1': Shade (with solid black) residues that match the Consensus exactly.

Divergence

Percent identity				
	1	2		
1		31.6	1	
2	100.0		2	
	1	2		

BM-HABP Fig.4.PRO TSG-6.PRO PubMed

Entrez

BLAST

OMIM

Taxonomy

Structure

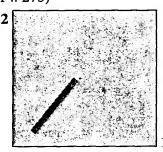
## **BLAST 2 SEQUENCES RESULTS VERSION BLASTP 2.1.2 [Oct-19-2000]**

Matrix BLOSUM62 gap open: 11 gap extension: 1

x\_dropoff: 50 expect: 10.000 wordsize: 3 Filter ✓ Align

**Sequence 1** lcllsèq\_1 **Length** 353 (1 .. 353) **Sequence 2** lcllseq\_2 **Length** 275 (1 .. 275)





NOTE: The statistics (bitscore and expect value) is calculated based on the size of nr database

Score = 107 bits (264), Expect = 5e-21 Identities = 45/104 (43%), Positives = 61/104 (58%)

Query: 52 DTTVGVFHLRSPLGQYKLTFDKAREACANEAATMATYNQLSYXQKAKYHLCSAGWLETGR 111 + GV+H + G+YKLT+ +A+ C E +ATY QL +K +H+C+AGW+ GR Sbjct: 32 EQAAGVYHREARAGRYKLTYAEAKAVCEFEGGRLATYKQLEAARKIGFHVCAAGWMAKGR 91

Query: 112 VAYPTAFASQNCGSGVVGIVDYGPRPNKSEMWDVFCYRMKDVNC 155 V YP NCG G GI+DYG R N+SE WD +CY C Sbjct: 92 VGYPIVKPGPNCGFGKTGIIDYGIRLNRSERWDAYCYNPHAKEC 135

SDJCE: 92 VGYPIVKPGPNCGFGKTGIIDYGIRLNRSERWDAYCINPHAREC 135

CPU time: 0.15 user secs. 0.03 sys. secs 0.18 total secs.

Gapped

Lambda K H

0.321 0.138 0.429

Gapped

Lambda K H

0.270 0.0470 0.230

Matrix: BLOSUM62

Gap Penalties: Existence: 11, Extension: 1

Number of Hits to DB: 748 Number of Sequences: 0 Number of extensions: 55

Number of successful extensions: 1
Number of sequences better than 10.0: 1

Number of HSP's better than 10.0 without gapping: 1

Number of HSP's successfully gapped in prelim test: 0
Number of HSP's that attempted gapping in prelim test: 0

Number of HSP's gapped (non-prelim): 1

```
length of query: 353
length of database: 3,171,650,076
effective HSP length: 58
effective length of query: 295
effective length of database: 3,171,650,018
effective search space: 935636755310
effective search space used: 935636755310
T: 9
A: 40
X1: 16 ( 7.4 bits)
X2: 128 (49.9 bits)
X3: 128 (49.9 bits)
S1: 41 (21.9 bits)
S2: 83 (36.7 bits)
```

#### EXHIBIT C

	GVFHLRSPLGQYKLTFDKAREACANEAATMATYNQ 		<del></del>
115	TAFASQNCGSGVVGIVDYGPRPNKSEMWDVFCYR	149	
	TAFASQNCGSGVVGIVDYGPRPNKSEMWDVFCYR	1156	

# CURRENT PROTOCOLS IN MOLECULAR BIOLOGY

YOLUME 1

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Ic. Harsh treatment: Pour several hundred milliliters of boiling 0.1% SDS onto the membrane. Cool to room temperature.

If a membrane is to be reprobed, it must not be allowed to dry out between hybridization and stripping. If it becomes dry, the probe may bind to the matrix.

 Place membrane on a sheet of dry Whatman 3MM filter paper and blot excess liquid with a second sheet. Wrap the membrane in plastic wrap and setup an autoradiograph.

If signal is still seen after autoradiography, rewash using harsher conditions,

3. The membrane can now be rehybridized. Alternatively, it can be dried and stored for later use.

Membranes can be stored dry between Whatman 3MM paper for several months at room temperature. For long-term storage, place the membranes in a desiccator at room temperature or 4°C.

#### REAGENTS AND SOLUTIONS

Aqueous prehybridization/hybridization (APH) solution

5x SSC (APPENDIX 2)

5× Denhardt solution (APPENDER 2)

1% (w/v) SDS

Add 100 µg/ml denatured salmon sperm DNA (see below) just before use

Alternatives to Denhardt solution and denatured salmon sperm DNA as blocking agents are listed in Table 2.10.5 (see discussion in critical parameters).

#### Denatured salmon sperm DNA

Dissolve 10 mg Sigma type III salmon sperm DNA (sodium salt) in 1 ml water. Pass vigorously through a 17-G needle 20 times to shear the DNA. Place in a boiling water bath for 10 min, then chill. Use immediately or store at -20°C in small aliquots. If stored, reheat to 100°C for 5 min and chill on ice immediately before using.

# Formamide prehybridization/hybridization (FPH) solution

5×SSE (APPENDIX2)

5× Denhardt solution (APPENDIX2)

50% (w/v) formamide

1% (w/v) SDS

Add 100 µg/ml denatured salmon sperm DNA (see above) just before use

Alternatives to Denhardt solution and denatured salmon sperm DNA as blocking agents are listed in Table 2.10.5 (see discussion in critical parameters).

Commercial formamide is usually satisfactory for use. If the liquid has a yellow color, deionize as follows: add S g of mixed-bed ion-exchange resin [e.g., Bio-Rad AG S01-X8 or 501-X8(D) resins] per 100 ml formamide, stir at room temperature for I hr, and filter through Whatman no. I paper.

- CAUTION: Formamide is a teratogen. Handle with care.

#### Labeling buffer

200 mM Tris-Cl, pH7.5

30 mM MgCl,

10 mM spermidine

#### Mild stripping solution

5 mM Tris-CL pH 8.0

2 mM EDTA

0.1× Denhardt solution (APPENDIX 2)

Hybridization Analysis of DNA Blots

## SDS electrophoresis buffer, 5× 15.1 g Tris base 72.0 g glycine 5.0 g SDS H<sub>2</sub>O to 1000 ml Dilute to 1x or 2x for working solution, as appropriate Do not adjust the pH of the stock solution, as the solution is pH &.3 when diluted. Store at 0° to 4°C until use (up to I month). SED (standard enzyme diluent) 20 mM Tris-Cl, pH 7.5 500 μg/ml bovine serum albumin (Pentax Fraction V) 10 mM 2-mercaptoethanol Store up to 1 month at 4°C Sodium acetate, 3 M Dissolve 408 g sodium acetate-3H<sub>2</sub>O in 800 ml H<sub>2</sub>O Add H2O to 1 liter Adjust pH to 4.8 or 5.2 (as desired) with 3 M acetic acid Sodium acetate buffer, 0.1 M Solution A: 11.55 ml giacial acetic acid/liter (0.2 M). Solution B: 27.2 g sodium acetate (NaC2H3O23H2O)/liter (0.2 M). Referring to Table A.2.2 for desired pH, mix the indicated volumes of solutions A and B, then dilute with H2O to 100 ml. (See Potassium acetate buffer recipe for further details.) Sodium phosphate buffer, 0.1 M Solution A: 27.6 g NaH2PO4H2O per liter (0.2 M). Solution B: 53.65 g Na<sub>2</sub>HPO<sub>4</sub>7H<sub>2</sub>O per liter (0.2 M). Referring to Table A.2.3 for desired pH, mix the indicated volumes of solutions A and B, then dilute with H2O to 200 ml. (See Potassium phosphate buffer recipe for further details.) SSC (sodium chloride/sodium citrate), 20x 3 M NaCl (175 g/liter) 0.3 M Na<sub>3</sub>citrate-2H<sub>2</sub>O (88 g/liter) Adjust pH to 7.0 with 1 M HCI STE buffer 10 mM Tris-Cl, pH 7.5 10 mM NaCl 1 mM EDTA, pH 8.0 TAE (Tris/acetate/EDTA) electrophoresis buffer Working solution, pH -8.5: 50x stock solution: 242 g Tris base 40 mM Tris-acetate 57.1 ml glacial acetic acid 2 mM Na<sub>2</sub>EDTA-2H<sub>2</sub>O 37.2 g Na<sub>2</sub>EDTA-2H<sub>2</sub>O H<sub>2</sub>O to 1 liter TBE (Tris/borate/EDTA) electrophoresis buffer 10× stock solution, 1 liter:

108 g Tris base (890 mM) 55 g boric acid (890 mM)

40 mi 0.5 M EDTA, pH 8.0 (20 mM)

Appendix 2

A.2.5